Prevalence of *Salmonella, Toxoplasma, Yersinia,* Hepatitis E and Porcine Reproductive and Respiratory Syndrome virus in UK pigs at slaughter

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Abstract
In 2006/7 the prevalence of *Salmonella* in UK slaughter pigs was found to be amongst the highest in Europe. In advance of the anticipated EU-wide National Control Programme for *Salmonella* in pigs, a UK-wide monitoring programme was set up by Defra and funded in collaboration with key Government and industry partners. The monitoring programme presented a cost-effective opportunity to concurrently collect data on other potentially zoonotic pathogens for which prevalence data is currently limited, including *Toxoplasma*, *Yersinia*, Hepatitis E virus (HEV) and Porcine Reproductive and Respiratory Syndrome virus (PRRSv). Antimicrobial resistance testing of *Campylobacter coli* isolates and testing for ESBL *E. coli* was also undertaken (details not reported here). This study will meet urgent evidence needs and improve our knowledge regarding potential risks to public health in the pig meat supply chain. Between January and April 2013, 600 slaughter pigs from 14 abattoirs throughout the UK were sampled. Sampling was weighted according to abattoir throughput. Sampling dates and pig carcasses were randomly allocated utilising the abattoir daily throughput as a sampling frame. Samples collected from each pig include a post-stun rectal swab (*Salmonella*), post-bleed serum samples (*Toxoplasma*, HEV and PRRSv), the caecum (*Salmonella*) and tonsils (*Yersinia* and PRRSv) at the evisceration point, and pre-chill carcase swabs (*Salmonella* and *Yersinia*). *Salmonella* isolation (ISO 6579), serotyping and phage typing, and the isolation of *Yersinia*, were undertaken at AHVLA. Plasma samples were tested by ELISA for antibodies to *Toxoplasma* (at the *Toxoplasma* Reference Unit), HEV (at Public Health England (PHE)) and PRRSv (at AHVLA), and also tested for HEV RNA (at PHE). Tonsil material from PRRSv-seropositive pigs was tested by PCR and used for virus sequencing (at AHVLA).

Introduction

Foodborne diseases are estimated to cost the UK nearly £1.5 billion per year (FSA, 2011). *Salmonella* is the second most common foodborne zoonosis, with 9,455 laboratory-confirmed cases in the UK in 2011 (EFSA, 2013). Control of *Salmonella* in the EU has thus far focused predominantly on poultry, with *Salmonella* National Control Programmes (NCPs) having been implemented in the various chicken and turkey sectors from 2007 (Defra, 2011). The success of the NCPs in poultry has resulted in a progressive reduction in the number of cases in people. However, the proportion of human cases attributed to pork and pork products appears to be rising.

In 2006/7, an EU baseline survey was conducted to assess the prevalence of *Salmonella* in pigs at slaughter (Commission Decision 2006/668). Levels in the UK were above the EU average; 40.4% (259/641) of pigs had evidence of current infection and/ or carcase contamination, with *Salmonella* isolated from 21.8% of lymph node samples, 15.1% of carcase swabs and 21.9% of caecal samples. A NCP for *Salmonella* in pigs is expected to commence in 2015 and reduction targets based on the EU mean prevalence are likely to be challenging for the UK pig industry. Since the last survey, efforts continued to reduce the prevalence of *Salmonella* in pigs. Therefore, a new monitoring programme to reassess the prevalence of infection was proposed. This presented an opportunity to simultaneously gather national-level prevalence data on other organisms to address additional knowledge gaps.

In a recent EFSA Opinion on pig meat inspection, *Toxoplasma gondii* was identified as one of the most significant foodborne public health hazards (EFSA, 2011). There are an estimated 350,000 toxoplasmosis cases in the UK each year, of which 10-20% are symptomatic (ACMSF, 2012). Despite pig meat being considered a high risk food for transmission, a recent report from the Advisory Committee on the Microbiological Safety of Food (ACMSF) noted that there is virtually no data concerning the prevalence in UK livestock (ACMSF, 2012).

In the EFSA Opinion, *Yersinia* was also deemed very important (EFSA, 2011). Yersiniosis was the fourth most frequently reported zoonosis in the EU in 2011 and infections are often acquired from raw or undercooked pig meat (EFSA, 2013). Consequently, EU

Member States have been advised to gather prevalence data for pigs at slaughter at regular intervals (EFSA, 2009).

The number of indigenous hepatitis E virus (HEV) infections in people in England have increased substantially recently. Pigs have been hypothesised to play a role due to the high prevalence of anti-HEV antibodies identified in previous surveys and the close phylogenetic relationship between HEV strains infecting humans and pigs. Pork products are unlikely to become laden with virus by faecal contamination, so it was pertinent to assess the quantity of pigs that are viraemic at slaughter from which products subsequently entering the food chain may include viraemic blood, such as muscle.

Porcine Reproductive and Respiratory Syndrome virus (PRRSv) can have a major economic impact on pig production. Infection can cause direct costs from loss of production, increased mortality and reproductive failure, and indirect costs associated with treatment, disease control, pig disposal costs and disrupted breeding programmes. Consequently, PRRSv has been targeted for control by the British Pig Executive (BPEX, 2011). Minimal data is presently available on seroprevalence in the UK, so the monitoring programme provided an ideal opportunity to determine how many pigs were potentially infectious at slaughter and to augment current data on PRRSv diversity.

A UK-wide multi-agency monitoring programme was launched to address these various evidence needs, involving collaboration between the Department for Environment, Food and Rural Affairs (Defra), the Department of Agriculture and Rural Development (DARD), the Food Standards Agency (FSA), the British Pig Executive (BPEX), Public Health England (PHE), Public Health Wales (PHW), the Veterinary Medicines Directorate (VMD) and the Animal Health and Veterinary Laboratories Agency (AHVLA). The study design was consistent, as far as possible, with the technical specifications of the EU baseline survey of 2006/7 to enable comparisons.

Methods

Sample size: The target sample size for the EU baseline survey was 600 pigs based on an estimated prevalence of 50% with an accuracy of 4% and 95% confidence (Commission Decision 2006/668). Utilising the same sample size would enable detection of a 20% overall reduction in *Salmonella* prevalence or a 40% reduction in carcase contamination (with 80% power and 95% confidence). An additional 10% (60 pig carcasses) were included as a contingency.

Participants: The target population was all slaughtered pigs (finishers and cull sows and boars) in the UK. The following animals were excluded: any carcase that was totally condemned; animals with a live weight <50kg; animals that had undergone emergency slaughter; and animals kept in the UK <3 months before slaughter. The largest capacity slaughterhouses in Great Britain (GB) and Northern Ireland (NI) were used, which together represented over 80% of all pigs slaughtered in the UK. The number of pigs sampled at each slaughterhouse was proportional to the annual throughput.

Sampling schedule: Samples were scheduled for collection between January and April 2013. The date of sampling and the specific pig carcase to sample on each occasion were randomly assigned by AHVLA utilizing the daily throughput as a sampling frame for the latter.

Sample and data collection: Samples were collected by trained staff at the FSA and DARD. Samples collected from each pig comprised of one post-stun rectal swab, two post-bleed EDTA whole blood samples, the caecum, heart, tongue and tonsils at the evisceration point, and two pre-chill carcase swabs. Samples were transported by courier (at 2-8°C) as soon as possible after collection. A standardised questionnaire was also completed at the time of sampling to gather epidemiological data, including information concerning the pig (e.g. age, weight) and abattoir processes (e.g. slaughter speed, scalding temperature).

Laboratory analysis: Bacteriological examination was carried out within 24 hours of receipt at the lab and 96 hours of sample collection.

Salmonella: Caecal contents, carcase swabs and rectal swabs were tested for *Salmonella* at AHVLA following the method in ISO6579 annex D, with pre-enrichment in Buffered Peptone Water, subculture to Modified Semi-Solid Rappaport-Vassiliadis medium then plating to Xylose-Lysine-Desoxycholate Agar and Novobiocin Brilliant Green Agar. Suspect colonies were confirmed serologically or biochemically. Positive isolates were serotyped according to the White-Kaufmann-Le Minor scheme and Typhimurium isolates were phage typed at the AHVLA national reference laboratory.
Yersinia: Carcase swabs and tonsils were tested for *Yersinia* at AHVLA via the cold enrichment method; samples were

stored at 2-8°C and inoculated onto *Yersinia* selective agar at weekly intervals for 3 successive weeks. Speciation was via colony morphology and API 20E biochemical strips.

PRRSv: EDTA plasma samples were tested for PRRSv antibodies by ELISA at AHVLA. Tonsil material from seropositive pigs was tested by PCR and a subset of viruses was sequenced.

Toxoplasma: EDTA plasma samples were tested for *Toxoplasma* antibodies using the Sabin-Feldman Dye Test at PHW. Heart and tongue tissue from seropositive pigs were stored for future investigations.

HEV: EDTA plasma samples were also tested for HEV antibodies by ELISA at PHE. In addition, HEV RNA levels were quantified using quantitative Taqman or hemi-nested PCR as appropriate (Garson *et al*., 2012; Jothikumar *et al*., 2006). Samples with sufficient viral loads were subject to sequence analysis in the ORF2 region.

Data collection and analysis: Data were registered on a survey specific MS Access database. Data cleaning and statistical analyses will be conducted in Stata 12.0. The overall prevalence of each organism will be calculated accounting for clustering of pigs within farms. These will be compared with previous abattoir surveys to investigate recent trends and the data for *Salmonella* will help develop the NCP in pigs. Agreement between sample types will be examined via kappa tests. Variation in regional prevalence will also be investigated. Co-infections will be explored. The questionnaire data will be used to investigate associations with the various microorganisms via multivariable logistic regressions models.

Results

The prevalence results from this unique multi-agency, multi-funded project should be available later this year. By combining numerous infectious agents into one monitoring programme, the project will be able to efficiently and simultaneously address urgent evidence needs and improve knowledge of numerous potential risks to public health in the pig meat supply chain. The survey has attracted considerable interest to date and the results are likely to be extremely useful to both the industry and public health bodies. The project has also enabled the strengthening of the collaborative ties between veterinary and public health organisations and the industry in the UK, which will prove beneficial for future research and surveillance and the implementation of the pig NCP.

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